BANANA GUARD

Protocols

PCR

Colony PCR

(Adapted from Thermo Scientific DreamTaq DNA polymerase product information)

- Gently vortex and briefly centrifuge all solutions after thawing.
- Place a thin-walled PCR tube on ice and add the following components for each 50 µl reaction:

COMPONENT	50 µl REACTION
10X DreamTaq Buffer	5 µl
dNTP Mix, 10 mM	1 µl
10 µM Reverse primer VR	0.5 µl
10 µM Forward primer VF2	0.5 µl
DreamTaq DNA Polymerase 5u/ µl	0.25 µl
Water, nuclease-free	to 49 µl
Template DNA	1 µl

- Gently vortex the samples and spin down.
- Place the reactions in a thermal cycler using the most appropriate program for the fragment that one aims to amplify. An standard example of a PCR run would be:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	15 min	1
Denaturation	95	30 sec	35
Annealing	60	30 sec	
Extension	72	2 min	
Final Extension	72	5 min	1

Elongation PCR

(*Adapted from New England Biolabs Q5 DNA polymerase product information)*

- Gently vortex and briefly centrifuge all solutions after thawing.
- Place a thin-walled PCR tube on ice and add the following components for each 50 µl reaction:

COMPONENT	50 µl REACTION
5X Q5 reaction buffer	10 µl
dNTP Mix, 10 mM	1 µl
10 µM Reverse primer VR	2.5 µl
10 µM Forward primer VF2	2.5 µl
Q5 high-fidelity	0.5 µl
Water, nuclease-free	to 50 µl
Template DNA	variable

- Gently vortex the samples and spin down.
- Place the reactions in a thermal cycler and use the most appropriate program depending on the desired amplification product.